noassay (EIA-2), which replaced EIA-1 in March 1992. Enzyme immunoassay 2 has improved specificity over EIA-1 with no loss of sensitivity, yielding fewer "false-positive" results, that is, reactivity without infection, based on follow-up studies of transfusion recipients and using "confirmatory" testing.

Investigational confirmatory techniques consist of an antibody detection using a modified Western blot (the recombinant immunoblot assay), a neutralization assay, and direct testing for HCV RNA by polymerase chain reaction. Blood specimens reactive by EIA-1 or EIA-2 have been shown to correlate with disease in the transfusion setting. Although the 40% to 70% falsely reactive rate with EIA-1 has been reduced significantly (to less than 30%) by the use of EIA-2, research efforts for improved "third-generation" techniques (still based on nonstructural proteins) are already under way. In addition, the control of specimen storage and handling is critical for valid testing.

More than 30 million units of donated blood have been screened with the EIA-1 anti-HCV test, resulting in the exclusion of between 120,000 and 330,000 (0.4% to 1.1%) blood donors who were unknowingly anti-HCV–reactive. Because nearly 50% of persons infected by HCV have asymptomatic but persistent liver damage, including chronic active hepatitis, cirrhosis, and even hepatocellular carcinoma, research to minimize or interrupt the natural course of this disease is indicated. Asymptomatic former blood donors provide an identified pool of potential subjects who would be directly benefited by such research efforts. Transfusion recipients of blood donated in 1992 can expect more than a 20-fold reduction in the risk of posttransfusion hepatitis because blood is screened for anti-HCV.

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Acquired Immunodeficiency Syndrome Vaccines

THE DEVELOPMENT OF AN effective vaccine against the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) is urgent and difficult. A successful vaccine must protect against cell-associated and cell-free virus at genital mucosal surfaces and in the bloodstream and must exert broad protection against the tremendous genetic variation exhibited by the virus. Because we do not understand the nature of protective immunity against HIV or have an animal model of HIV-1–induced disease, we must rely heavily on animal lentiviral infections to guide us toward a deeper understanding of the pathogenesis of the virus and the development of an effective AIDS vaccine.

The picture from the animal lentivirus models, however, is still unclear. Using recombinant HIV-1 envelope vaccines, a few chimpanzees have been protected against infection with a low dose of the homologous strain (IIIB) of HIV-1, apparently by the induction of a threshold level of neutralizing antibodies. Similar recombinant envelope vaccine approaches, however, have not been generally effective in protecting against challenge infection in the other animal lentivirus models. In these other animals, the best protection has been achieved with vaccines made of inactivated whole virus or attenuated live virus, but the mechanism of protection remains uncertain and such whole-virus approaches are not considered safe or practical for humans.

The shortfalls of these experimental vaccines are important: they require at least three injections over several months; the immunity is rather short-lived (less than a year); and the protection does not extend to intravenous challenge with higher doses of cell-free virus, to intravenous infection with cell-associated virus, or to infection across an intact genital mucosa. None of the experimental HIV-1 vaccines have yet been tested in chimpanzees against widely divergent HIV-1 strains such as occur in nature. In general, the immune protection elicited by the experimental vaccines in animals appears to convey a complete block to infection after challenge. This type of "sterilizing immunity" may be required of a vaccine to prevent later viral activation and disease, but it may also explain why the protection is so limited and why antiviral cytotoxic T-lymphocyte activity is not usually induced by these vaccines. The induction of strong $CD8^+$ cytotoxic T-lymphocyte activity is almost certainly a requirement of any truly efficacious HIV vaccine, and this may depend on a live vector or an attenuated live virus approach.

In the United States, seven different phase 1 and 2 trials, using candidate HIV-1 recombinant envelope vaccines and adjuvants, are under way in more than 500 volunteer uninfected people. Two trials use core peptides (p17 and p24), and one trial uses inactivated (gamma irradiation and β-propiolactone) envelope-deficient whole HIV-1. In general, these immunogens have proved safe and immunogenic, but they have induced mainly type-specific neutralizing antibody of short duration (less than 6 months) and low titer (less than that seen in natural infection) and no $CD8^+$ cytotoxic T-lymphocyte activity. Live vaccinia vaccine (HIV-recombinant envelope) priming followed a year later by boosting with an HIV-envelope subunit (expressed in baculovirus) has had some encouraging results, but the vaccinia vector would not be safe for large efficacy trials unless further attenuated. Also, live vaccinia vectors would not be effective in vaccinia-immune persons. None of these phase 1 or 2 HIV-1 vaccine candidates have elicited the strong, durable, broadly reactive humoral and cytotoxic T-lymphocyte immune responses needed to justify larger-scale efficacy trials.

On the optimistic side, several hundred HIV-seropositive but immunocompetent persons who have received the HIV gp160 vaccine after infection have generally responded favorably by generating antibodies and T cells that recognize new antigenic determinants in the envelope. Insufficient time has elapsed to determine whether this treatment will suppress viral replication and delay the onset of AIDS. Evaluating the safety and immunogenicity of various HIV-1 immunogens given to HIV-1–infected subjects will help determine the suitability of these materials for prophylactic vaccination.

The criteria for selecting vaccine candidates for efficacy trials are unsettled, and we must decide on the end points to be used: preventing infection or delaying or preventing disease. For the latter, we need to define surrogate markers to predict disease, such as quantitative virus load, that can be measured early after infection. It is hoped that further preclinical developments in the animal models using different viral immunogens, different adjuvants, different routes of antigen presentation (such as mucosal), novel live vectors

(such as bacille Calmette-Guérin and adenovirus), and novel recombinant DNA constructs may yet lead to promising efficacy results as a basis for large-scale testing. In the meantime, high-risk uninfected cohorts are now being recruited and the infrastructure put in place in the United States and abroad for efficacy tests of one or more of the most promising candidate HIV-1 vaccines that may become available in several years. The first vaccines used may be only partially effective, but their early introduction might still prevent many infections, reduce viral transmission, delay disease, and provide valuable information that could facilitate future vaccine development. Multiple vaccines that are tailor-made for the predominant HIV strain(s) circulating at different global sites may need to be tested simultaneously. A long-term commitment has been made to vaccine development; this must include realistic expectations and both preclinical and clinical trials.

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Laboratory Evaluation of Inherited Thrombotic Disorders

RECENT ADVANCES IN THE diagnosis of and therapy for thrombotic disease have focused attention on the consulting role of clinical pathologists in the laboratory evaluation of coagulation disorders. Patients referred for evaluation usually have venous thromboembolism, but some may have arterial thrombotic events. Inherited disorders that can be tested for in a coagulation laboratory may result from abnormalities in inhibitors of activated coagulation factors (antithrombin III, protein C, protein S), impaired clot lysis (dysfibrinogenemia, plasminogen deficiency, tissue-plasminogen activator deficiency, excess plasminogen activator inhibitor), or a metabolic disorder associated with vascular disease and thrombosis (homocystinuria).

Although only about 30% of patients with recurrent thrombosis have an inherited disorder identified, the yield of this evaluation can be improved by restricting it to young patients with recurrent thrombosis or patients with thrombosis and a positive family history. The laboratory evaluation should be deferred until two to three months after the acute event (to avoid acute-phase changes that may obscure a correct diagnosis) and after the patient has discontinued anticoagulant therapy. Many laboratories offer both functional and immunologic assays for evaluating these disorders. Because thrombotic disease may result from either quantitative deficiency or a qualitative abnormality of these proteins (for example, antithrombin III or protein C) and immunologic assays may yield normal results in patients with dysfunctional proteins, it is preferable to do functional assays that detect both types of disorders. Appropriate specimen collection, timing, and processing are critical, especially for fibrinolytic assays (tissue-plasminogen activator, plasminogen activator inhibitor). Deficiencies of proteins C and S and antithrombin III are thought to be the most common causes of inherited thrombosis. Previous studies indicating that fibrinolytic abnormalities were common causes of recurrent thrombosis have been recently challenged. A metabolic disorder, heterozygous homocystinuria, has been increasingly associated with arterial vascular disease and should be considered in middle-aged patients with premature arterial thrombosis.

One approach in the laboratory investigation of recurrent thrombosis is to first exclude acquired causes of hypercoagulability—hyperlipidemia, lupus anticoagulant, or malignancy. The presence of venous thrombosis should be initially evaluated with a functional protein C assay and a free protein S antigen assay. If the results of these tests are normal, a functional assay for antithrombin III can be done, followed by testing for dysfibrinogenemia. If the test results are normal, the fibrinolytic system could be evaluated.

Unexplained arterial thrombosis in young patients can be evaluated with an assay of plasminogen activator inhibitor activity, whereas middle-aged patients with premature arterial thrombotic events should be tested for heterozygous homocystinuria using a methionine loading test.

The importance of laboratory monitoring of anticoagulation has been emphasized by studies showing the clinical benefit of adequate heparin and warfarin therapy in treating thromboembolism. Because the responsiveness of commercial laboratory-activated partial thromboplastin time reagents may vary substantially, each laboratory should establish its therapeutic heparin range (activated partial thromboplastin time ratio, 1.5 to 2.5), so that it is equivalent to a heparin level of 200 to 400 U per liter (0.2 to 0.4 U per ml) by protamine titration. Despite the large body of published evidence indicating the clinical importance of using the international normalized ratio format in reporting laboratory prothrombin times, many coagulation laboratories do not use this format, leading to inappropriate anticoagulant therapy for many patients. Increased educational efforts will be necessary to inform practitioners and pathology personnel of the importance of rigorous laboratory control of anticoagulation.

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Enhancing Antibody and Its Role in Acquired Immunodeficiency Syndrome

ANTIBODIES AGAINST VIRAL proteins have traditionally been considered beneficial to the host by preventing infection. This principle has been the mainstay of modern vaccine development. It is now known, however, that certain antibody responses may not benefit the host but may, indeed, benefit the virus. One such phenomenon, antibody-dependent enhancement, increases the infectivity of a variety of viruses from several different families. Antibody-dependent enhancement can occur when nonneutralizing antibodies bind to viral surface proteins. The Fc portion of this antibody can then be bound by Fc receptors on macrophages. Viruses that are resistant to lysosomal degradation can then infect macrophages by this route. A second mechanism has been described whereby nonneutralizing antibodies bind to viral